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FILE NO.: A-69164/DJB/JJD

TELECOPIER NUMBER: 1-703-308-0294

FROM:

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NUMBER OF PAGES: 12 (including transmittal sheet) If you do not receive clear copies of any pages, counting Transmittal Page as Page 1, please let us know.

DATE & TIME SENT: May 31, 2001

TRANSMITTED BY:

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RETURN TEXT TO: MONICA May 31, 2001

Form 25
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PATENT
Attorney Docket No.: A-69164/DJB/JJD
Genencor No.: GCL 266-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	VALLE, et al.	Examiner:	Prouty, R.
		Group Art Unit:	1652
Serial No.:	08/940,692		
Filed:	30 September 1997		
For:	APPLICATION OF GLUCOSE TRANSPORT MUTANTS FOR PRODUCTION OF AROMATIC PATHWAY COMPOUNDS		

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence, including listed enclosures (if any), is being sent by facsimile transmission to (703) 308-0294 to the attention of Examiner Prouty and addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231 on May 31, 2001.

Signed: Monica Carlos
Monica Carlos

AMENDMENT AND RESPONSE

Assistant Commissioner
for Patents
Washington, D.C. 20231

Sir:

This is submitted as a supplement to the amendment filed July 6, 2000 and is responsive to the Office Action mailed July 3, 2000. While no fee is believed to be due, the Commissioner is authorized to charge any additional fees including extension fees or other relief which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-69164/DJB/JJD).

Please enter the amended claim set and consider the remarks herein.

23. (Thrice Amended) A mutant host cell having a metabolic pathway which uses PEP as a precursor or intermediate of metabolism, said host cell characterized by:

(a) being phenotypically Pts-/glu+;

(b) requiring galactose permease activity to transport glucose; and

(c) having a specific growth rate on glucose as a sole carbon source of at least $0.4h^{-1}$.

24. A mutant host cell of Claim 23 comprising recombinant DNA coding for one or more of the enzymes selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase such that the mutant host cell expresses transketolase, transaldolase or phosphoenolpyruvate synthase at enhanced levels relative to wild-type host cells.

25. (Once amended) A mutant host cell of Claim 23 further comprising mutations in the pykA and/or pykF genes in said host cell.

26. (Once amended) A mutant host cell of Claim 24 further comprising mutations in the pykA and/or pykF genes in said host cell.

27. (Thrice Amended) A method for increasing PEP availability [into] to a biosynthetic or metabolic pathway of a host cell, the method comprising:

culturing a host cell mutant characterized by:

having a Pts-/glu+ phenotype;

requiring galactose permease activity to transport glucose; and

having a specific growth rate on glucose as a sole carbon source of at least $0.4h^{-1}$;

in the presence of an appropriate carbon source, wherein said host cell mutant utilizes PEP as a precursor or intermediate of metabolism.

28. A method of Claim 27 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of *ptsI*, *ptsH* and *crr*.

29. A method of Claim 27 further comprising modifying the selected host cell to introduce therein recombinant DNA coding one or more of the enzymes selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase such that the mutant host cell expresses transketolase, transaldolase or phosphoenolpyruvate synthase at enhanced levels relative to wild-type host cells.

30. A method of Claim 27 further comprising modifying the selected host cell to reduce or eliminate pyruvate kinase activity in said host cell.

31. A method of Claim 30 wherein pyruvate kinase activity is reduced or eliminated in the host cell by introducing a mutation in DNA encoding one or more of the sequences coding for pyruvate kinase, pyruvate kinase promoter region and other regulatory sequences controlling expression of pyruvate kinase.

33. (Once amended) A method of Claim 42 wherein the DNA used to transform the host cell encodes one or more enzyme(s) selected from the group consisting of DAHP synthase, DHQ synthase, DHQ dehydratase, shikimate dehydrogenase, shikimate kinase, EPSP synthase and chorismate synthase.

34. (Once amended) A method of Claim 42 further comprising transforming the host

cell with recombinant DNA coding one or more enzyme(s) selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase so that said enzyme is expressed at enhanced levels relative to wild-type host cells.

35. A method of Claim 33 further comprising transforming the host cell with recombinant DNA coding one or more enzyme(s) selected from the group consisting of transketolase, transaldolase and phosphoenolpyruvate synthase so that said enzyme is expressed at enhanced levels relative to wild-type host cells.

36. (Once amended) A method of Claim 42 wherein the desired compound is selected from the group consisting of tryptophan, tyrosine and phenylalanine.

37. A method of Claim 36 wherein the desired compound is tryptophan and the host cell is transformed with DNA coding one or more gene(s) selected from the group consisting of *aroG, aroA, aroC, aroB, aroL, aroE, trpE, trpD, trpC, trpB, trpA* and *tktA* or *tktB*.

38. (Twice Amended) A method for obtaining a Pts-/glucose+, galactose permease requiring-mutant cell, the method comprising:

(a) selecting a host cell which utilizes a phosphotransferase transport system;

(b) mutating the host cell whereby the phosphotransferase transport system is inactivated;

(c) culturing the mutant host cell using glucose as a carbon source; and

(d) selecting a mutant host cell which grows on glucose at a specific growth rate of at least 0.4 h^{-1}.

39. (once amended) A method of Claim 38 wherein the mutant cells are selected due

to a specific growth rate on glucose of about 0.8 h^{-1}.

40. The mutant cell of Claim 23 having a specific growth rate on glucose as a sole carbon source of about 0.8h^{-1}.

41. The mutant cell of Claim 23 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of ptsI, ptsH and crr.

42. A method for enhancing production of a desired compound in a modified host cell, said host cell in its unmodified form being capable of utilizing a phosphotransferase transport system for carbohydrate transport, the method comprising,

(a) culturing a modified host cell with an appropriate carbon source, said modified host cell characterized by having:

(i) a Pts-/glu+ phenotype;

(ii) requiring galactose permease activity to transport glucose;

(iii) a specific growth rate on glucose as a sole carbon source of at least 0.4h^{-1}; and

(iv) utilizing PEP as a precursor or intermediate of metabolism,

said modified host cell further comprising recombinant DNA encoding one or more enzyme(s) catalyzing reactions in the pathway of biosynthetic production of said desired compound in said modified host cell; and

(b) optionally recovering said compound.

43. (Amended) The method of Claim 42, wherein the modified host cell has a specific growth rate on glucose as a sole carbon source of about 0.8h^{-1}.

44. (Amended) The method of Claim 42 wherein the Pts- phenotype is caused by the deletion or inactivation of all or substantially all of one or more gene(s) selected from the group consisting of ptsI, ptsH and crr.

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45. The method of Claim 38 wherein mutating the host cell is by inactivating the phosphotransferase transport system.

46. The method of Claim 45 wherein said inactivating is by deleting part or all of gene(s) selected from the group consisting of ptsI, ptsH and crr.

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